PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Miyamoto et al.

Group Art Unit: 1636

Serial No.: 10/534,424

Examiner: Dunston, Jennifer Ann

Filed: May 10, 2005

Docket No.: 1680/7

Confirmation No.: 4728

For: TRANSCRIPTION REGULATOR ZHX3

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DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

1. I, Kazuya Yamada, am a co-inventor of the subject matter disclosed and claimed in the subject above captioned U.S. patent application, which upon information and belief claims priority to PCT Application No. PCT/JP03/009164 filed July 18, 2003, which upon information and belief claims priority to Japanese Application No. 2002-366512 filed December 18, 2002.

2. I have had the opportunity to review the Official Action mailed on October 18, 2007 from the U.S. Patent and Trademark Office for the above-referenced subject U.S. patent application.

3. Attached hereto as **Exhibit A** is a true and accurate hard copy of experimental data generated at Matsumoto University, which I am familiar with and have had the opportunity to review. The data in Exhibit A demonstrates that ZHX3 (SEQ ID NO: 1) can act as a transcriptional repressor. Specifically, ZHX3 can repress transcription of both type II hexokinase (HKII) and pyruvate kinase M (PKM) genes.

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: March 13, 2008 By: Kazuya Yamada

EXHIBIT A

TEST REPORT

Matsumoto University
Kazuya Yamada

In order to examine the function of ZHX3 in PKM and HKII gene expression, promoter assays were conducted by using SL2 cells. This cell line is devoid of endogenous NF-Y, Sp1, and the members of the ZHX family. Two luciferase reporter plasmids were prepared, which contain the nucleotide sequence between –287 and +46 of the rat *PKM* gene promoter and –157 and +147 of the rat *HKII* gene promoter, respectively.

(1) The promoter activity of the rat *PKM* gene was synergistically increased by co-transfection of both Sp1- and NF-Y-expression vectors in SL2 cells with a calcium phosphate method (*J. Biol. Chem.* 275, 18129-18137, 2000). The activity was decreased by a co-transfection with a ZHX3 expression vector as shown in attached Figure 1.

(2) The rat HKII promoter contains multiple NF-Y-binding sequences. When the HKII gene reporter plasmid was co-transfected with expression vectors of NF-Y, the promoter activity was increased as shown in attached Figure 2. The activity was also decreased by a co-transfection with ZHX3 expression vector as shown in attached Figure 2.

These results indicate that ZHX3 can repress both PKM and HKII promoter activities.

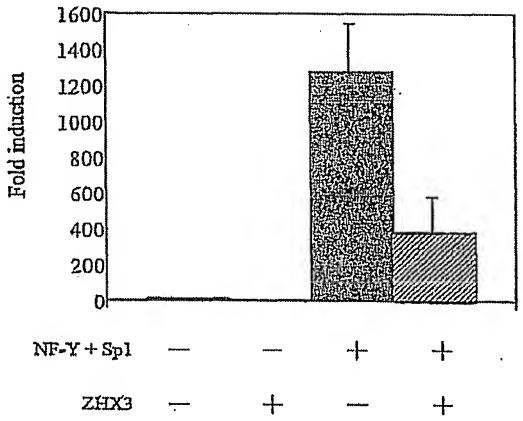

Fig. 1 ZHX3 represses promoter activity of the *PKM* gene stimulated by both NF-Y and Sp1 in *Drosophila* SL2 cells

Two μg of pMPK/Luc287 reporter plasmid was co-transfected with 100 ng of pPac-β-Gal, 100 ng of pPac or 25 ng of pPacNF-YA, 25 ng of pPacNF-YB, 25 ng of pPacNF-YC and 25 ng of pPac-Sp1 with or without 200 ng of pPac-ZHX3 into SL2 cells by a calcium phosphate method. Total DNA amount (2.4 μg) was adjusted by the addition of the pPac plasmid. Each column and error bar represents the mean and standard error of at least four separate transfection experiments. The data are presented as fold stimulation where the value of luciferase activity normalized to β-galactosidase activity for the reporter alone is set at 1.0.